

Multigene analysis of the catfish genus *Trichomycterus* and description of a new South American trichomycterine genus (Siluriformes, Trichomycteridae)

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<http://zoobank.org/5F7E526A-C810-4025-870D-21C995FE2C81>

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Abstract

Received 18 September 2018

Accepted 10 November 2018

Published 22 November 2018

Academic editor:

Peter Bartsch

Key Words

Phylogeny

Molecular Systematics

Classification

Ostariophysi

Trichomycterinae

Biodiversity

Trichomycterus comprises about 170 valid species, but its monophyly has been challenged in the last decades. Bayesian Inference and Maximum Likelihood analyses comprehending mitochondrial genes COI and CYTB and nuclear genes GLYT, MYH6 and RAG2 from 71 Trichomycterinae terminal taxa and eight outgroups were performed. The analyses highly supports a clade containing *Trichomycterus nigricans*, the type species of the genus, and several other congeners endemic to eastern and northeastern Brazil, herein considered as the genus *Trichomycterus*, the sister clade the southern Brazil and adjacent areas clade; the latter clade comprises two subclades, one comprising species of the genus *Scleronema* and another comprising species previously placed in *Trichomycterus*, herein described as a new genus. *Cambeva* **gen. n.** is distinguished from all other trichomycterines by the presence of a bony flap on the channel of the maxillo-dentary ligament, the interopercle shorter than the opercle, a deep constriction on the basal portion of the antero-dorsal arm of the quadrate, absence of teeth in the coronoid process of the dentary, the maxilla shorter than the premaxilla, the cranial fontanel extending from the the medial posterior of frontal to the medial region of supraoccipital, and absence of the postorbital process of the sphenotic-prootic-pterosphenoid.

Introduction

Siluriformes (catfishes) is among of the most diverse vertebrate orders, with about 3700 species in 39 families (Nelson et al. 2016, Frick et al. 2018). Trichomycteridae, the second most diverse catfish family, with about 300 species (Frick et al. 2018), is characterized by having a modified opercular system, involving the presence of odontodes in the interopercular and opercular bones (Baskin 1973, de Pinna 1992, 1998). It occurs between southern Central America, in Costa Rica, and southern South America, in Patagonia (Baskin 1973, de Pinna 1998), exhibiting an impressive species diversity and endemism around higher elevations (de Pinna 1992). Presently, Trichomycteridae comprises eight subfamilies: Trichomycterinae Bleeker, 1858, Vandelliinae Bleeker,

1862, Stegophilinae Günther, 1864, Tridentinae Eigenmann, 1918, Glanapteryginae, Myers 1944, Sarcoglanidinae Myers & Weitzman, 1966, Trichogeninae Isbrücker, 1986 and Copionodontinae de Pinna, 1992. Trichomycterinae is the most species rich subfamily, with 220 species in eight genera: *Bullockia* Arratia, Chang, Menu-Marque & Rojas, 1978; *Eremophilus* Humboldt, 1805; *Hatcheria* Eigenmann, 1909; *Ituglanis* Costa & Bockmann, 1993; *Rhizosomichthys* Miles, 1943; *Scleronema* Eigenmann, 1917; *Silvinichthys* Arratia, 1998; and *Trichomycterus* Valenciennes, 1832 (Frick et al. 2018).

Among the Trichomycterinae, *Trichomycterus* is the most species diverse genus, comprising about 170 valid species (Frick et al. 2018) and occurring in a geographical range coincident to that described for the whole family (Arratia 1998; de Pinna 1998). Inhabiting mainly high-al-

titude rapid stream rivers, a remarkable feature occurring among species of *Trichomycterus* is the ability to climb waterfalls (Eigenmann 1918, Arratia 1983) with the support of the interopercle and opercle odontodes, in an ‘elbowing’ behaviour (de Pinna 1992, Adriens et al. 2010).

Since Baskin (1973), monophyly of *Trichomycterus* has been challenged by several authors (Arratia et al. 1978, de Pinna 1989, Arratia 1990a, Costa and Bockmann 1993, Datovo and Bockmann 2010, Ochoa et al. 2017), but its phylogenetic status still remains unclear. Morphological taxonomic studies could not find unique diagnostic characters for *Trichomycterus* (de Pinna 1989), whereas some species traditionally placed in *Trichomycterus* have been reallocated in other trichomycterine genera (e.g. Costa and Bockmann 1993, Arratia 1998, Henschel et al. 2017). Furthermore, recent molecular analyses have suggested that species still today placed in *Trichomycterus* do not form a monophyletic group (Ochoa et al. 2017).

In addition to the uncertain status of *Trichomycterus*, two other trichomycterine genera, *Scleronema* and *Ituglanis*, have unclear relationships. They are often considered more related to non-trichomycterine trichomycterids (Myers and Weitzman 1966, de Pinna 1989, Costa and Bockmann 1994, DoNascimento 2015), but more recent studies indicated that they are members of the Trichomycterinae (Datovo and Bockmann 2010, Henschel et al. 2017, Ochoa et al. 2017).

The objective of this paper is to provide the more inclusive molecular based phylogenetic analysis of *Trichomycterus*, assigning the phylogenetic position of its type species, *Trichomycterus nigricans* Valenciennes, 1832 and describing a new genus, sister to *Scleronema*, that was hidden under the lack of consistent information about *Trichomycterus* relationships.

Materials and methods

Taxon sampling

Specimens were euthanized by sub-merging them in a buffered solution of tricaine methanesulphonate (MS-222) at a concentration of 250 mg/L, for a period of 10 min, following the guidelines of the Journal of the American Veterinary Medical Association (AVMA Guidelines) (Leary et al. 2013) and European Commission DGXI consensus for fish euthanasia (Close et al. 1996, 1997). Molecular data were obtained from specimens fixed and preserved in absolute ethanol. Specimens used for morphological comparisons were fixed in formalin for a period of 14 days and then transferred to 70% ethanol. All the collected material was deposited in the fish collection of Institute of Biology, Federal University of Rio de Janeiro (UFRJ).

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from muscle tissues using the DNeasy Blood & Tissue Kit (Qiagen), according to the manufacturer’s protocol. The analyses included a set of partial sequences of four nuclear genes: recombination activating 2 (RAG2), myosin heavy chain 6 (MYH6), SH3 and PX domain containing 3 (SH3PX3), glycosyltransferase

(GLYT); and partial sequences of two mitochondrial encoded genes: cytochrome c oxidase I (COI) and cytochrome b (CYTB). Amplification of the target DNA fragments was made through the polymerase chain reaction (PCR) method, using the following primers: Cytb Siluri F, Cytb Siluri R, L5698-ASN, H7271-COI (Villa-Verde et al. 2012), Glyt_F577, Glyt_F559, Glyt_R1562, Glyt_R1464, myh6_F459, myh6_F507, myh6_R1322, myh6_R1325, SH3PX3_F461, SH3PX3_R1303, SH3PX3_F532, SH3PX3_R1299 (Li et al. 2007); RAG2 MCF, RAG2 MCR (Cramer et al. 2011), MHRAG2-F1 and MHRAG2-R1 (Hardman & Page, 2003). Double-stranded PCR amplifications were performed in 60 µl reactions with reagents at the following concentrations: 5× GreenGoTaq Reaction Buffer (Promega), 3.2 mM MgCl₂, 1 µM of each primer, 75 ng of total genomic DNA, 0.2 mM of each dNTP and 1 U of standard Taq polymerase or Promega GoTaq Hot Start polymerase. The thermocycling profile was as follows: initial denaturation for 2 min at 94–95 °C; 35 cycles of denaturation for 1 min at 94 °C, annealing for 1 min–90 s at 48.0–64.0 °C and extension for 1–2 min at 72 °C; and terminal extension for 4 min at 72 °C. Negative controls were used to check on contaminations. The PCR products were then purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems). Cycle sequencing reactions were performed in 20 µl reaction volumes containing 4 µl BigDye, 2 µl sequencing buffer 5× (Applied Biosystems), 2 µl of the amplified products (10–40 ng), 2 µl primer and 10 µl deionized water. The thermocycling profile was: (1) 35 cycles of 10 seconds at 96 °C, 5 seconds at 54 °C and 4 minutes at 60 °C.

Phylogenetic analyses

In-group included seven subfamilies of Trichomycteridae lineages. Since this study was directed to searching for relationships and monophyly of *Trichomycterus*, the analysis included a total of 50 terminal taxa presently placed in this genus, as well as 21 terminal taxa belonging to the trichomycterine genera *Bullockia*, *Eremophilus*, *Ituglanis* and *Scleronema*. Out-group selection was directed to sample representatives of other lineages of the Trichomycteridae, comprising six terminal taxa representing each of the remaining subfamilies, as well as representatives of other families of the Loricarioidea, including the nematogeniid *Nematogenys inermis* Guichenot, 1848 and the loricariid *Pareiorhina rudolphi* (Miranda-Ribeiro, 1911), in which the analyses were rooted. A list of specimens and its respective GenBank accession numbers is provided in Suppl. material 1. Sequences were aligned and edited in Mega 7 software (Kumar et al. 2016) using the ClustalW algorithm (Chenna et al. 2003). Gaps were considered as informative characters. The data set was partitioned by gene, and the best-fit evolutionary model selection was performed under the Akaike information criteria in the software jmodeltest 2 (Darriba et al. 2012) for each partition. Bayesian Inference (BI) and Maximum Likelihood (ML) analyses were conducted using the softwares Mrbayes 3.2 (Ronquist et al. 2012) and Garli 2.0 (Zwickl 2006), re-

spectively. The BI analysis was conducted with the following parameters: two independent Markov chain Monte Carlo (MCMC) runs of two chains each for 20 million generations, with a tree sampling frequency of every 100 generations. The convergence of the MCMC chains and the proper burn-in value were assessed by evaluating the stationary phase of the chains using tracer v. 1.5 (Rambaut et al. 2013). The BI final consensus tree and its Bayesian posterior probabilities were generated with the remaining tree samples after removing the first 25% samples as burn-in. To test the support of the nodes in the ML analysis, 1,000 bootstrap (Felsenstein 1985) replicates were made.

Morphological comparisons

Morphological comparisons were focused on the external morphology and osteological features of cleared and stained specimens prepared according to Taylor and Van

Dyke (1985). Terminology for osteological nomenclature follows Arratia (1998). Measurements and counts follow Barbosa and Costa (2003). A list of material analysed appears in Suppl. material 2.

Results

Phylogenetic analyses

The concatenated matrix comprised 4380 bp after alignment (522 bp for COI, 857 for CYTB, 890 for GLYT, 542 for MYH6, 884 for RAG2 and 680 for SH3PX3). The best-fit evolutionary models found are shown in Suppl. material 3. The best log-likelihood score for the ML analysis was -31473.209522 and the mean for BI was -lnl -32013.626.

Both analyses exhibit similar topologies (Figs 1, 2). In all analyses, monophyly of the Trichomycterinae was high-

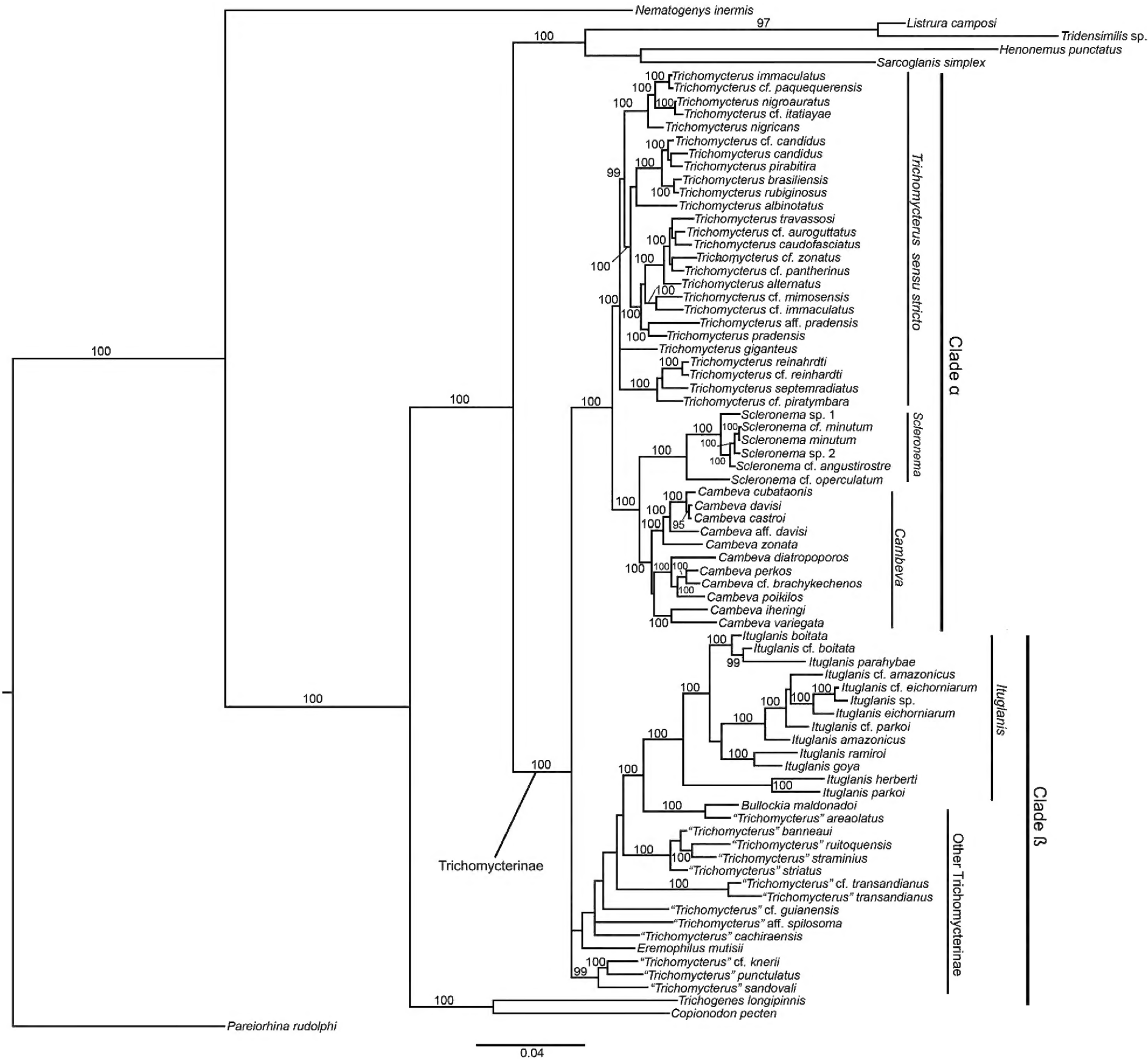


Figure 1. Phylogenetic positioning of *Cambeva* among the Trichomycteridae, inferred by Bayesian Inference from the analysis of molecular data, total of 4380 bp comprising segments of nuclear genes for GLYT, MYH6, RAG2 and SH3PX3 and the mitochondrial genes COI and CYTB. Numbers on each node represent posterior probabilities.

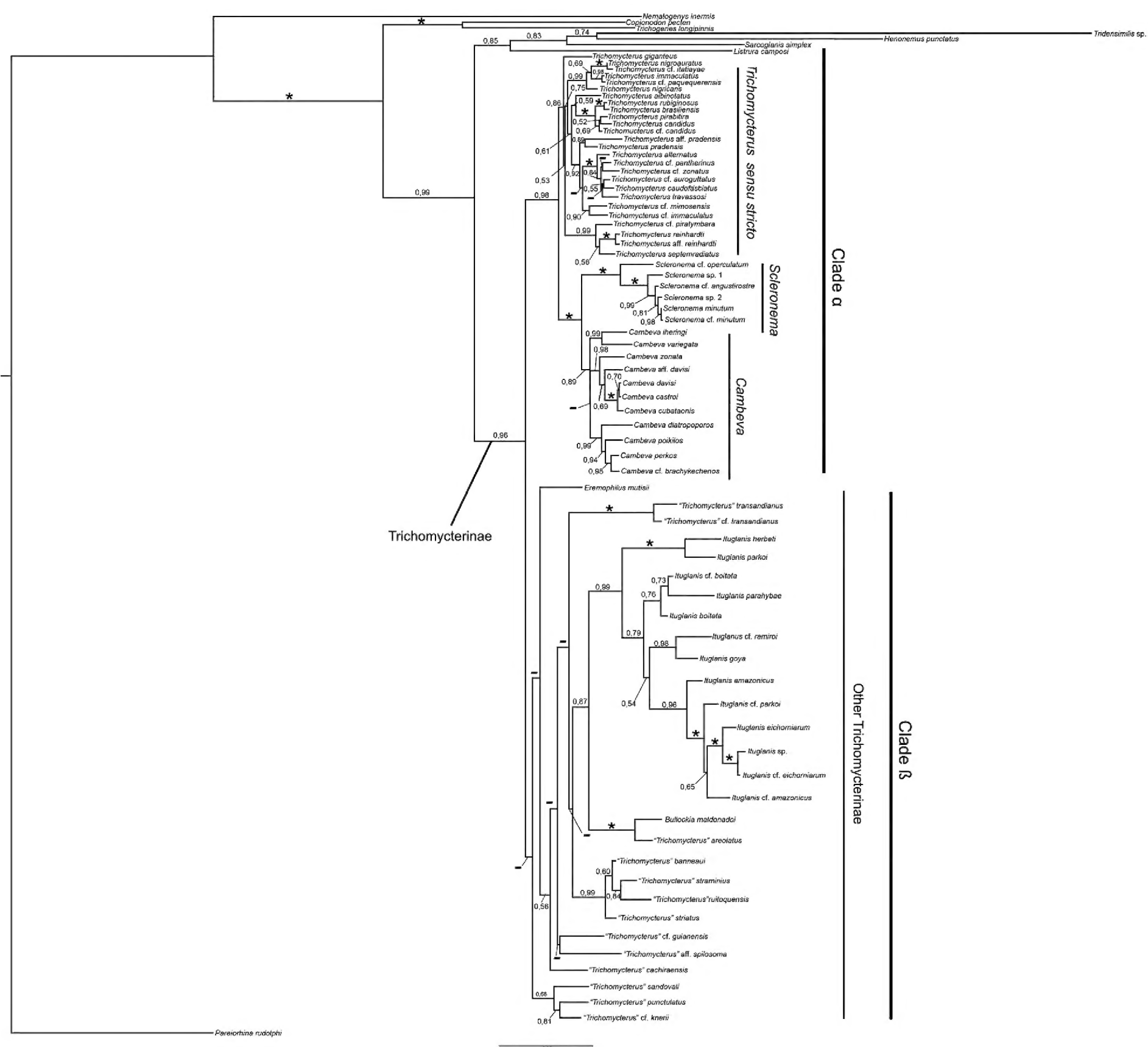


Figure 2. Phylogenetic positioning of *Cambeva* among the Trichomycteridae, inferred by Maximum Likelihood from the analysis of molecular data, total of 4380 bp comprising segments of nuclear genes for GLYT, MYH6, RAG2 and SH3PX3 and the mitochondrial genes COI and CYTB. Numbers on each node are bootstrap percentages of the Maximum Likelihood analysis; asterisks indicate maximum support value and hyphen values under 50.

ly supported, but nominal species of *Trichomycterus* did not form a single monophyletic group. Two major clades were found in Trichomycterinae. The first one, the trichomycterine clade a, is highly supported in both analyses (Figs 1, 2) and comprises species geographically restricted to an area encompassing eastern, south-eastern and southern Brazil, which have been traditionally placed in two genera, *Scleronema* and *Trichomycterus*. Both analyses highly corroborate three major subclades (Figs 1, 2), one comprising *T. nigricans*, the type species of *Trichomycterus*, and other congeners, therefore herein recognised as the true *Trichomycterus*; another corresponding to the genus *Scleronema*; and another clade, sister to *Scleronema* and comprising several nominal species of *Trichomycterus*, herein recognised as a new genus (see taxonomic accounts below).

The second trichomycterinae group, the clade b, is weakly supported in the BI analysis (Fig. 1), but not sup-

ported in the ML analysis (Fig. 2). It includes taxa endemic to a broad geographical area, including the Andean region, Amazon, and Patagonia, formally placed in the genera *Bullockia*, *Eremophilus*, *Ituglanis* and *Trichomycterus*, which is herein graphed “*Trichomycterus*” by being distantly related to that clade including the type species of the genus. Relationships among basal lineages of the clade b were weakly supported (BS < 50; BI < 95).

Taxonomic accounts

***Cambeva* gen. n.**

<http://zoobank.org/C26BEA49-7714-44FB-957B-678D8C6C9DCC>

Type species. *Pygidium davisii* Haseman, 1911 (Fig. 3)

Diagnosis. *Cambeva* is similar to *Scleronema* and distinguished from all other genera of the Trichomycter-

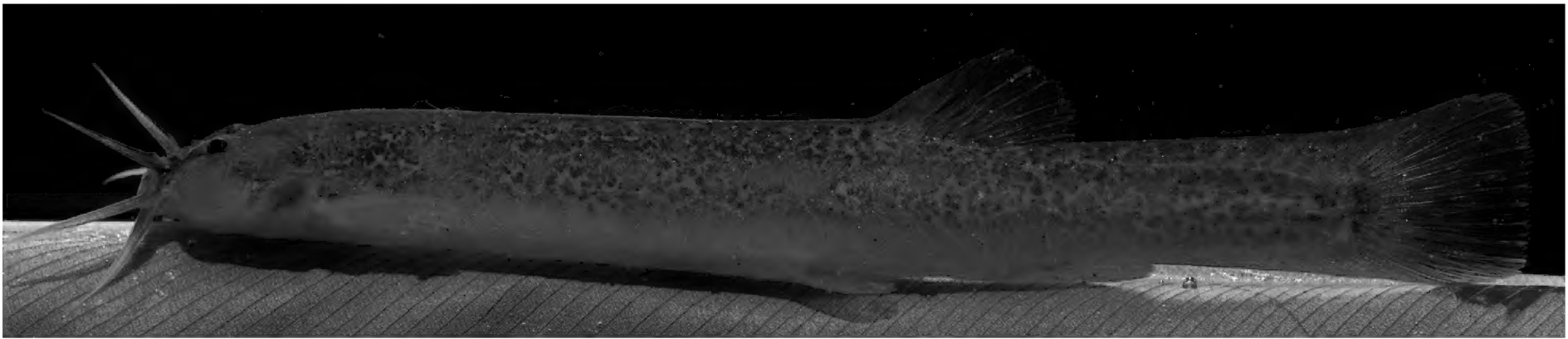


Figure 3. *Cambeva davisi*, topotype, UFRJ 9759, 67.6 mm SL; Brazil: Paraná: Balsa Nova. Photograph by A. M. Katz.

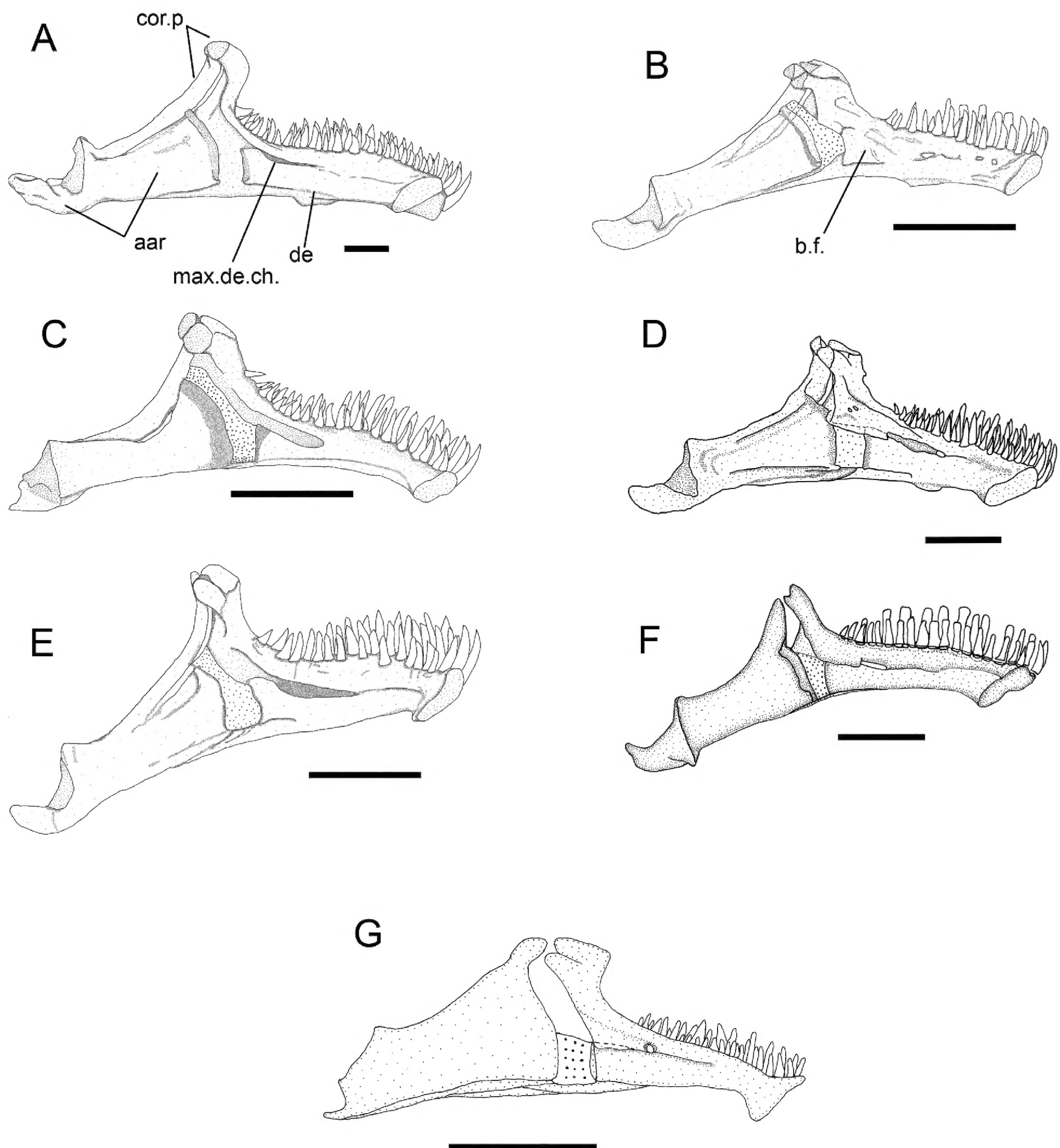


Figure 4. Left lower jaw in medial view. **A** *Trichomycterus giganteus*, UFRJ 5732, paratype. **B** *Cambeva davisi*, UFRJ 10713. **C** *Trichomycterus alternatus*, UFRJ 9900. **D** *Cambeva zonata*, UFRJ 11900. **E** *Trichomycterus brasiliensis*, UFRJ 4834. **F** *Cambeva brachykechenos*, UFRJ 10586. **G** *Scleronema operculatum*, UFRJ 11856. Scale bar: 1 mm aar, anguloarticulo-retroarticular; corp.p, coronoid process; de, dentary; max.de.ch., maxillo-dentary channel; b.f. bone flap.

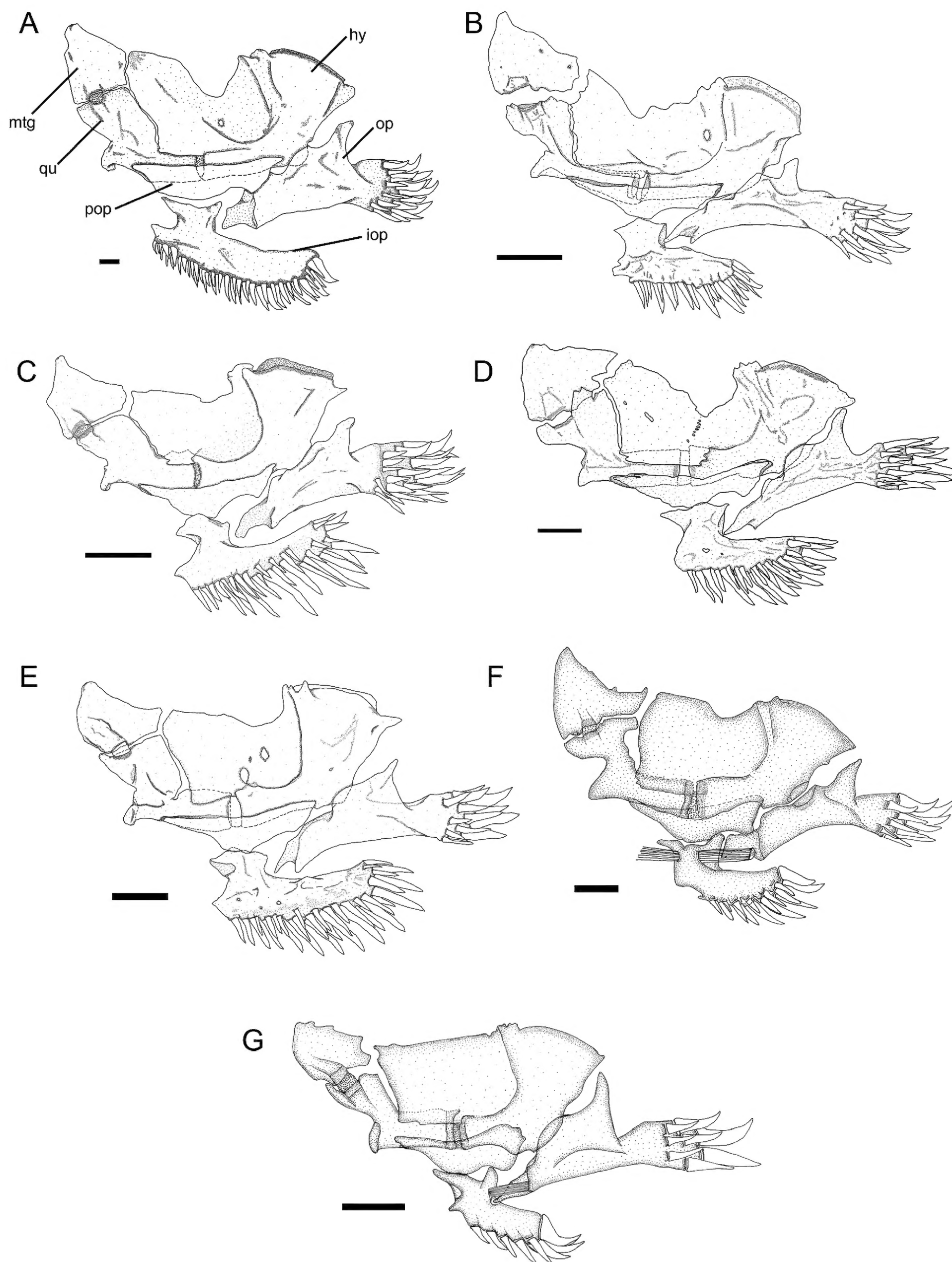


Figure 5. Left suspensory in dorsal view. **A** *Trichomycterus giganteus*, UFRJ 5732, paratype. **B** *Cambeva davisi*, UFRJ 10713. **C** *Trichomycterus alternatus*, UFRJ 5673. **D** *Cambeva zonata*, UFRJ 11900. **E** *Trichomycterus brasiliensis*, UFRJ 4834. **F** *Cambeva brachykechenos*, UFRJ 10586. **G** *Scleronema operculatum*, UFRJ 11856. Scale bar: 1 mm hy, hyomandibula; iop, interopercle; mtg, metapterygoid; op, opercle; pop, preopercle.

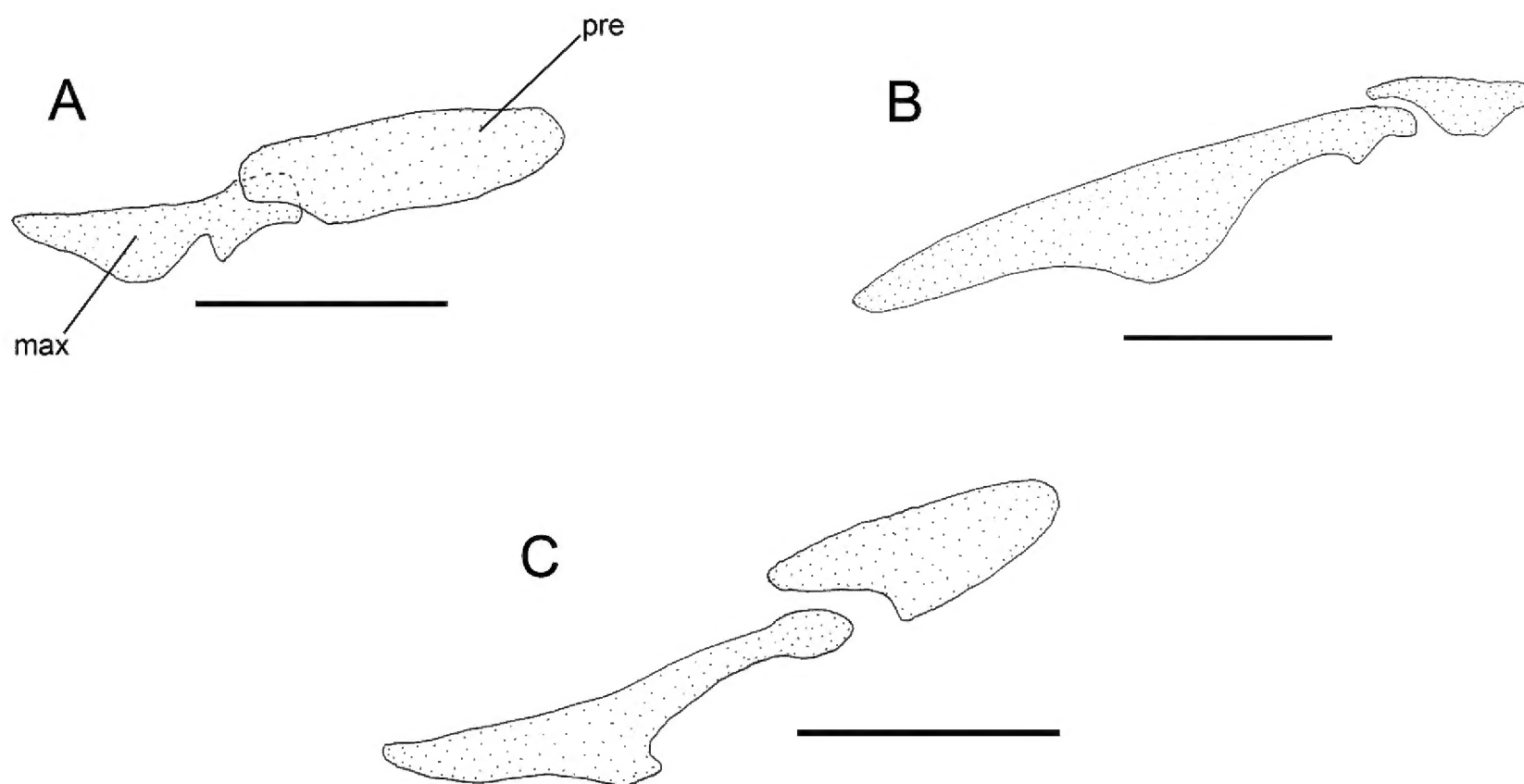


Figure 6. Dorsal view of premaxilla and maxilla. **A** *Cambeva davisi*, UFRJ 10713 **B** *Scleronema operculatum*, UFRJ 11856. **C** *Scleronema* sp1., UFRJ 10645. Scale bar: 1 mm max, maxilla; pre, premaxilla.

nae by the presence of a bony flap covering the posterior segment of the maxillo-dentary ligament channel in the dentary (Fig. 4B, D, F, G vs. flap absent, Fig. 4A, C, E; see also Arratia 1998: fig. 9a). *Cambeva* also differs from most Trichomycterinae except *Scleronema* by the presence of a short interopercle, shorter than the opercle (Fig. 5B, D, F, G; vs. longer, Fig. 5A, C, E; see also Arratia 1998: fig. 9b, Schaefer & Fernández 2009: fig. 3, Adrians et al. 2010: fig. 5 c,d), a deep constriction on the basal portion of the antero-dorsal arm of the quadrate in lateral view, its width less than 50% quadrate width at its dorsal limit (Fig. 5B, D, F, G; vs. more than 50%, Fig. 5A, C, E; see also Arratia 1990b: fig. 10a-c, Arratia 1998: fig. 8a, b), and absence of teeth in the coronoid process of the dentary (vs. presence), (Fig. 4B, D, F, G, Arratia 1998: fig. 9a). *Cambeva* differs from *Scleronema* by having the maxilla shorter than the premaxilla (maxilla with 30-60% length of premaxilla (Fig. 6A) vs. 90% or more (Fig. 6B, C)), absence of a transverse skin fold between the anterior nostrils and the maxillary barbel (vs. presence), 4–6 abdominal vertebrae (vs. 1–3), and absence of a fleshy projection posteriorly extending to opercular patch of odontodes (vs. presence). *Cambeva* is also distinguished from *Ituglanis* by possessing the cranial fontanel extending from the medial posterior of frontal to the medial region of supraoccipital (vs. restricted to the posterior region of the parieto-supraoccipital) (Costa & Bockmann 1993: fig. 3A), absence of the postorbital process of the sphenotic-prootic-pterosphenoid (vs. presence of an anteriorly directed postorbital process on the sphenotic-prootic-pterosphenoid) (Costa & Bockmann 1993: fig. 3A, B) and a smooth medial rim of the autopalatine (vs. deeply concave) (Costa & Bockmann 1993: Fig. 6).

Included species. *Cambeva davisi* (Haseman, 1911), *Cambeva iheringi* (Eigenmann, 1917), *Cambeva zonata* (Eigenmann, 1918), *Cambeva brachykechenos* (Ferrer & Malabarba, 2013), *Cambeva castroi* (de Pinna, 1992), *Cambeva cubataonis* (Bizerril, 1994), *Cambeva diatropoporos* (Ferrer & Malabarba, 2013), *Cambeva poikilos* (Ferrer & Malabarba, 2013), *Cambeva variegata* (Costa, 1992). Other species, not included in the molecular analysis but exhibiting generic morphological diagnostic character states and congruent geographical distribution are: *Cambeva stawiarski* (Miranda Ribeiro, 1968), *Cambeva balios* (Ferrer & Malabarba, 2013), *Cambeva concolor* (Costa, 1992), *Cambeva crassicaudata* (Wosiacki & de Pinna, 2008), *Cambeva diabola* (Bockmann, Casatti & de Pinna, 2004), and *Cambeva naipi* (Wosiacki & Garavello, 2004). The following species, were not examined or lack the necessary osteological information on their original descriptions, but have the general external appearance and occur in the same basins that *Cambeva* is distributed, so they are tentatively included in the new genus: *Cambeva paolence* (Eigenmann 1917), *Cambeva guaraquessaba* (Wosiacki, 2005), *Cambeva igobi* (Wosiacki & de Pinna, 2008), *Cambeva mboyacy* (Wosiacki & Garavello, 2004), *Cambeva pascuali* (Ochoa, Silva, Silva, Oliveira & Datovo, 2017), *Cambeva perkos* (Datovo, Carvalho & Ferrer, 2012), *Cambeva plumbea* (Wosiacki & Garavello, 2004), *Cambeva tropeiro* (Ferrer & Malabarba, 2011), *Cambeva tupinamba* (Wosiacki & Oyakawa, 2005), and *Cambeva ytororo* (Terán, Ferrer, Benitez, Alonso, Aguilera & Mirande, 2017).

Distribution. Species of *Cambeva* gen. n. occur in the Paraná, São Francisco, Ribeira de Iguape, and Uruguay river basins, as well as in smaller isolated coastal river basins of south-eastern and southern Brazil.

Etymology. *Cambeva*, probably derived from the Tupi-Guarani, is a popular name for trichomycterid fishes in southern and south-eastern Brazil. Gender: feminine.

Discussion

Monophyly of Trichomycterinae

Since the first objective phylogenetic analysis of the Trichomycteridae by Baskin (1973), monophyly of the Trichomycterinae has been challenged by several authors. Besides not finding unique diagnostic features for the subfamily, supposed derived traits shared by some trichomycterines and species of other trichomycterid subfamilies made uncertain the position of some trichomycterine members. de Pinna (1989) suggested that the trichomycterine genus *Scleronema* is more closely related to the subfamily Sarcoglanidinae than to other trichomycterines, as already discussed by Myers & Weitzman (1966), due to the common possession of an enlarged maxilla; Arratia (1990) also reported a maxilla enlargement for the trichomycterine genera *Bullockia* and *Hatcheria*, besides species of ‘*Trichomycterus*’. Our results clearly indicate that taxa having long maxilla (e.g., sarcoglanidines, *Bullockia*, *Scleronema*) do not form a monophyletic group. Considering the psammophilic habits exhibited by species of Sarcoglanidinae and *Scleronema*, with specimens digging into sand or gravel (Zuanon and Sazima 2004, Schaefer et al. 2005, AM Katz and WJEM Costa pers. obs.), the long maxilla exhibited by these taxa may be interpreted as an adaptive convergence generating homoplasies in both groups.

Costa and Bockmann (1993) considered *Ituglanis* and *Scleronema* more closely related to the TSVSG clade, comprising the Tridentinae, Sarcoglanidinae, Vandelliinae, Stegophilinae and Glanapteryginae (Costa and Bockmann 1994), than to other trichomycterids based on a thin tip of the lateral process of the urohyal and a slightly reduced interopercular patch of odontodes. However, as noted by subsequent authors (e.g. Fernández and de Pinna 2005; Datovo and Bockmann 2010; the present study) other trichomycterines also exhibit in some extent those character states. More recently, Datovo and Bockmann (2010), in their myological analysis of trichomycterids, found evidence indicating that *Ituglanis* and *Scleronema* are more closely related to other Trichomycterinae than to the TSVSG clade. A similar result has been proposed in molecular phylogenies (e.g. Henschel et al. 2017, Ochoa et al. 2017). In our multigene analysis, using a broader genetic sample, the phylogenetic position of *Ituglanis* and *Scleronema* within a monophyletic Trichomycterinae is highly corroborated (Figs 1, 2). *Ituglanis*, with species distributed in the main tropical and subtropical Cis-Andean river basins of South America, did not appear as closely related to *Scleronema*, but sister to a clade comprising Andean, Patagonian and Amazon species of ‘*Trichomycterus*’ (Figs 1, 2).

Monophyly of Cambeva

All species here included in *Cambeva* were previously placed in *Trichomycterus*, instead of being considered as more closely related to *Scleronema* (Eigenmann 1918, Bizerril 1994, Wosiacki and Garavello 2004). Datovo and Bockmann (2010) examined myological characters of several trichomycterids, among which were two species of *Cambeva*, *C. davisii* and *C. stawianski*, which according those authors share a unique apomorphic condition consisting of the extensor tentaculi originating exclusively from the ethmoidal region of the neurocranium. We have searched this derived condition in several species of *Trichomycterus* and *Cambeva* herein examined (see Suppl. material 2), but it was only found in species endemic to some coastal rivers of southern Brazil, and to the Iguazu, Ribeira do Iguape and Parapanema river basins (*C. castroi*, *C. cubataonis*, *C. davisii*, *C. naipi* and *C. zonata*), suggesting that this derived condition is synapomorphic for a subclade of *Cambeva*.

Cambeva is here supported by high values of bootstrap and posterior probability as monophyletic and sister to *Scleronema* (Figs 1, 2). However, in spite of the clade comprising *Cambeva* and *Scleronema* being diagnosed by morphological synapomorphies, we did not find unique character states for *Cambeva*, which is only distinguishable from other trichomycterine genera by a combination of derived and primitive morphological character states (see Diagnosis above).

Acknowledgements

We are grateful to E. Caramaschi and V. Abilhoa for the loan and donations of specimens, and to F. Pereira, E. Henschel, P. Bragança, P. Amorim, P. Vilardo, O. Simões, R. Marques, T. Barros and G. Beltrão for assistance during field expeditions. This paper benefited from suggestions provided by Peter Bartsch and Felipe Ottoni. This study was supported by CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico - Ministério de Ciência e Tecnologia) and FAPERJ (Fundação de Amparo à Pesquisa do Estado do Rio de Janeiro).

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Supplementary material 1

GenBank accession codes

Authors: Axel Makay Katz, Maria Anais Barbosa, José Leonardo de Oliveira Mattos, Wilson José Eduardo Moreira da Costa

Data type: MS Word document

Explanation note: Terminal taxa for molecular phylogeny and respective GenBank accession numbers.

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Link: <https://doi.org/10.3897/zse.94.29872.suppl1>

Supplementary material 2

Occurrences and morphological data

Authors: Axel Makay Katz, Maria Anais Barbosa, José Leonardo de Oliveira Mattos, Wilson José Eduardo Moreira da Costa

Data type: MS Word document

Explanation note: List of material examined for the analysis of morphological characters.

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Link: <https://doi.org/10.3897/zse.94.29872.suppl2>

Supplementary material 3

Genetics data

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Data type: MS Word document

Explanation note: Best-fit evolutionary models found for each mitochondrial gene and for each codon position of nuclear genes.

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